Prevalence of Contagious Bovine Pleuropneumonia in Western Bahr El Ghazal State, South Sudan

By

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DEDICATION

To My Beloved Africa First,

My Family and

My Dearest Friends

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ABSTRACT

Contagious Bovine Pleuropneumonia (CBPP) is considered one of the most serious diseases that threaten the livestock sector in Western Bahr El Ghazal State, Republic of South Sudan.

This study was designed to assess the prevalence, distribution, related risk factors, and economic importance of CBPP by serological test. Participatory Disease Search (PDS) methods were used to detect clinical cases so as to provide useful information that would support the surveillance and control strategies of the disease in Western Bahr El Ghazal State (Wau and Jur River counties).

A total of 291 serum samples were collected from two groups of cattle (143 from group A, and 148 from group B); group A were cattle that have been infected with CBPP for the last 2-3 years, and group B were those cattle that were infected with CBPP less than 1 year or had clinical signs of CBPP. These sera were collected from different locations in Western Bahr El Ghazal State (43 herds, 5 payams and two counties). The samples were tested using Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA). The results showed that the rates of positive reactors were 13.2% for group A and 12.8% for group B.

The competitive ELISA was more sensitive in the diagnosis of CBPP and is a useful tool to be applied in the field.

The Participatory Disease Search (PDS) result among cattle keepers indicated that these communities have valuable knowledge about CBPP (they know clinical signs, post mortem findings and seasonality of the disease) which would support credible surveillance and control strategies for the disease.

The study suggested that CBPP has a seasonal pattern of prevalence in Western Bahr El Ghazal State, being much more prevalent during the rainy season. Therefore, movement control and vaccination programme are very important to be applied in the beginning of the rainy season.

المستخلص

يعتبر مرض ذات الرئه السارى فى الابقار واحد من اكثر الامراض خطورة المهددة لقطاع الماشية فى ولاية غرب بحر الغزال، جمهورية جنوب السودان.

هدفت هذه الدراسة لمعرفة معدل إنتشار وتوزيع وعوامل الخطورة ذات العلاقة وألاهمية ألاقتصادية لمرض ذات الرئه السارى فى الابقار وذلك عن طريق الاختبارات المصلية . تم إستخدام طرق البحث عن المرض بمشاركة المجتمعات لتحديد الحالات المرضية لتساعدنا فى الحصول على المعلومات اللازمة التى تدعم التقصى المرضى و استراتجيات السيطرة على المرض فى ولاية غرب بحر الغزال (مقاطعتى واو ونهر الجور).

جمعت 291 عينة مصل من مجموعتين من الابقار (143 من مجموعة أ و148 من مجموعة ب) مجموعة أ هى الابقار التى كانت مصابة بمرض ذات الرئه السارى فى الابقار قبل 2-3 سنوات ومجموعة ب هى الابقار التى تمت إصابتها قبل سنة أو لديها أعراض مرض ذات الرئه السارى فى الابقار. جمعت هذه العينات من مناطق مختلفة داخل ولاية غرب بحر الغزال (43 قطيع، 5 وحدات إدارية و مقاطعتين). اختبرت العينات بإستعمال إختبار الاليزا التنافسى (c- ELISA)، واوضحت النتائج بان نسبة التفاعلات الايجابية كانت 13.2% لمجموعة أ و 12.8% لمجموعة ب.

دلت النتائج على ان إختبار الاليزا التنافسى اكثر نجاحا فى تشخيص مرض ذات الرئه السارى فى الابقار يمكن إجراؤه حتى الحقل.

دلت المعلومات المستخلصة من مربيى الماشية عبر البحث عن المرض بمشاركة المجتمعات بان لهذه المجموعات الرعوية حصيلة معرفية بيطرية قيمة عن مرض ذات الرئه السارى فى الابقار(الالمام التام بالاعراض، الصفات التشريحية و موسم حدوث المرض) ومفيدة فى مجال إستيراتجيات المسح والسيطرة على المرض.

إقترحت الدراسه أن لمرض ذات الرئه السارى فى الابقار بولاية غرب بحر الغزال سلوك موسمى، حيث يكون معدل الاصابة أعلى خلال موسم الامطار منه خلال موسم الصيف. لذلك فالسيطرة على حركة الابقار وتطبيق برنامج التطعيم قبل هطول الامطار ذات أهمية قصوى.

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CHAPTER ONE

INTRODUCTION

Contagious Bovine Pleuropneumonia (CBPP) (lung sickness) is an acute, sub-acute or chronic disease of cattle and water buffalo. CBPP is caused by *Mycoplasma mycoides subsp. mycoides* small colony variant, *Mmm sc type* (Masiga and Domenech, 1995). The disease mainly affects the mucous membranes and joints. Infections with *Mycoplasama* spp. are invariably associated with respiratory and urogenital tracts, udder and eye. CBPP is one of the most important diseases of cattle, being contagious and devastating. It affects all ages causing respiratory signs in adult cattle and arthritis in calves. Chronic carriers are apparently healthy animals that have a localized focus of infection sequestrated in a fibrous capsule in their lungs. Such animals are always referred to as lungers. The incubation period of the disease is between 3 and 6 weeks and may reach up to 3 months.

In the acute form, there is fever lasting 3 to 10 days, anorexia, decrease in milk production, severe depression, and rapid labored abdominal breathing. This is soon followed by dry coughing and apparent chest pain, arch back, abducted elbows and extended neck. There may be nasal discharge and frothy saliva accumulated around the mouth. In the case of per acute form, which occurs during outbreaks, the animals die without showing any clinical signs (FAO, 2002). In sub-acute and chronic forms, clinical signs are milder and may not be detected. There may be an intermittent fever, loss of condition and respiratory signs following vigorous exercise.

The disease is transmitted by direct contact between infected and susceptible cattle, by infective aerosols from exhaled air. Transmission cannot occur

over distances greater than 100 m and some authors state a maximum of 20 m (Newton and Norris, 2000; Thiaucourt *et al.*, 2004a). The disease is not transmitted through contact with excreta, animal housing and equipment or vehicles previously occupied by infected animals (Turner, 1954; Hudson, 1971; Newton and Norris, 2000; Thiaucourt *et al.*, 2004a). In addition, cattle products (beef, milk and hides) are highly unlikely to transmit CBPP. Infection may also be passed on during mating since *Mycoplasma mycoides* subsp. *mycoides* small colony can be present in semen, but it is questionable whether it would then reach the respiratory tract (Masiga *et al.*, 1972; Gonçalves, 1994; Stradaioli *et al.*, 1999). Certain conditions under which cattle are closely herded together favour rapid spread of the disease. Chronically infected and symptomless animals play an important role in the persistence and spread of the disease (FAO, 2003).The most characteristic post-mortem finding in the acute form of the disease is presence of clear or turbid yellow or greenish-yellow fluid in the thoracic cavity, which varies in amount and may contain flakes. There may also be a thick layer of fibrinous deposit over the parietal and visceral pleura, which may, or may not be accompanied by hepatization of the lung.

The interlobular septa are distended with lymph fluid (Liyoid, 1971). In the late or chronic cases, necrotic portions of the lungs are walled off by fibrous tissue capsule, forming a sequestrum (Hudson, 1971). Sequestra vary in size from a centimeter in diameter to 20 or 30 cm in their greatest diameter. Adhesions of the lung to the thoracic wall may happen; Mediastinal and bronchial lymph nodes are swollen and oedematous. Demarcated renal infracts are clearly visible when the kidney capsule is striped off. Lesions in calves are often restricted to joints (Moulton *et al;* 1956).

CBPP is currently a disease of Africa, where it is regarded as one of the most serious transboundary animal diseases (in East, West, Central and part of the south Africa) with the exception of south Africa, Malawi, Mozambique, Zimbabwe and north Namibia in addition to north Africa and Madagascar Island (FAO 2002b). The disease has been reported in Central Europe and Italy in 1993 and Spain in 1994. In 2005, Portugal was free from CBPP. In Asia, CBPP has recently been reported in Bangladesh (FAO 2002).

CBPP is currently a disease of major concern throughout sub-Saharan Africa. It has occurred recently in both northwestern and southern Zambia, threatening the disease-free countries to the south (OIE, 2005a), while in central, eastern and western Africa the extent of endemic areas continues to increase (AU–IBAR, 2003a).

CBPP is of economic importance especially in Sudan where it causes decrease in production and high mortality rate, which adversely affect the export of Sudanese cattle to foreign markets (Abdulla, 1975). Mortality rate is 50% while the morbidity rate may reach 100%.

Objectives

The objectives of the study are:

1. To determine the prevalence of CBPP among cattle in different locations in Western Bahr El Ghazal State
2. To assess the extent to which carrier animals (lungers) may be responsible for occurrence of clinical CBPP.
3. To recommend credible surveillance and control strategies for CBPP in the study area.

CHAPTER TWO

LITERATURE REVIEW

2. 1. The disease

Contagious bovine pleuropneumonia (CBPP) is an infectious and highly contagious disease of cattle and water buffaloes (Hutyra *et.al*., 1938, Turner; 1937, Liyoid; 1970 and Hudson; 1971). It is considered one of the diseases amongst the most important infectious diseases of cattle. CBPP is caused by *Mycoplasma mycoides* subspecies *mycoides* (bovine biotype) SC (small colony) (Turner; 1959, Hudson; 1971 and OIÈ, 2002).

2. 2. History of the disease

2. 2. 1. Disease in the World

It is believed that in the 4th century BC Aristotle referred to the CBPP in his *History of Animals* (Newton and Norris, 2000). Epidemics were recorded throughout the middle ages (i.e. from about 1200 AD) and in Europe, concentrated mainly in the western alpine region. However, the disease commenced to spread in the 16th century and became a world-wide distribution only during the second half of the 19th century because of increased international trade in live cattle.

The first ‘modern’ description of the disease from Italy was provided by Gallo in 1564 (Thiaucourt *et al.*, 2004a) and the Napoleonic Wars (1800–1812) played an important role in acceleration of the disease spread across Europe and thus, it was reported in Britain in 1840 (Newton and Norris, 2000).

CBPP has occurred in most parts of the world at some time, with the exception of South America and Madagascar (Thiaucourt et al., 2004a); however, it is not known whether it spread from Europe.

A molecular epidemiological study has shown three lineages of the causative organism, *Mycoplasma mycoides* subspecies *mycoides*, small colony (*Mmm*SC) in Africa (Lorenzon *et al*., 2003). This finding indicates that CBPP probably occurred in the northern reaches of sub-Saharan Africa prior to the introduction of the disease to South Africa in 1854 by one or two bulls imported from the Netherlands (Henning, 1956; Thiaucourt *et al.*, 2004a).

The disease was reported in the United States of America in 1843 (Jasper, 1967), and it's eradication from the United States of America, Canada and most of Europe was achieved in the late 19th century through movement control and slaughter of clinical cases (Provost *et al.*, 1987) before vaccines and laboratory tests became available. However, the disease persisted in parts of Europe (e.g. France, Italy, Portugal, Spain, Germany and Austria) until quite recently. Eradication would not have been possible without laboratory testing because cases were difficult to detect clinically (Nicholas *et al.*, 1996).

CBPP spread to Australia through cattle imported from England in 1858 (Turner, 1959), and from Australia the infection was taken to Asia at the beginning of the 20th century. Australia eradicated the disease in 1973 after an intensive campaign of more than 10 years.

After virtual elimination from Europe in the 19th century the disease reappeared in Portugal and Spain in 1951 and 1957, respectively and Poland in 1936 (Viettoz, 1969). The later cases of outbreaks were reported in southern

France in (1973; Anon, OIE; 1974). In Italy the disease reappeared in 1990 but was eliminated by 1993 (Coetzer *et.al*., 1994).

CBPP has been reported in recent times from Assam in India, Bangladesh and Myanmar. Sporadic outbreaks have been recognized in the Middle East and Asia, probably derived from importation of cattle from Africa (Nicholas, and Bashiruddin, 1995). The rarity of pastoralism in Asia (in contrast to Africa, where CBPP is predominantly endemic to pastoralist herds) is a possible explanation. However, CBPP occurred in Mongolia, a country known for its pastoralists, up to 1972 (OIE, 2005a).

The disease still exists in most African countries south of Sahara, Angola, India and China (Hudson; 1971).

2. 2. 2. Disease in Africa

The disease was introduced into South Africa in 1854 through importations of cattle from the Netherlands, and from there it spread to other countries in the region. The origin of the disease in Central, West and East Africa is obscure. It has been suggested that the infection was introduced by zebu cattle when they first migrated to the African continent (Thiaucourt, Van der Lugt & Provost 2004).

There is a strong possibility that CBPP was introduced into East Africa from India by the army of Field Marshal Napier when he invaded Ethiopia in 1867-1868. However, Zimbabwe eradicated the disease in 1904, South Africa did so in 1924 and Botswana completed eradication in 1939. The infection was reintroduced into Botswana in 1994. North African countries have been infected only on a sporadic basis, the most recent being Egypt in 1972. However, Egypt rapidly eradicated the disease (Masiga *et al.*, 1996).

CBPP is currently a disease of major concern throughout sub-Saharan Africa and it is considered one of the most serious transboundary cattle diseases after Rinderpest in Africa (OAU-IBAR, 1990). About 26 countries were infected during at least some of the period between 1996 and 2003: Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Mali, Mauritania, Namibia, Niger, Nigeria, Rwanda, Somalia, Sudan, Tanzania, Togo, Uganda and Zambia (OIE, 2005a)

The only African countries to have claimed eradication of CBPP in recent times, either formally through the OIE or by informal declaration, are Botswana and Senegal. Botswana 'stamped out' an outbreak in Ngamiland in the northwest of the country in 1995 by slaughter the clinical cases (Mullins *et al.*, 2000). Additional methods of control were also introduced (e.g. construction of fences) but these had limited success (Scott Wilson and the Environment & Development Group, 2000). Senegal did so through vaccination programme and has claimed freedom from CBPP from 1997 to 2003 (OIE, 2005a).

Zonal freedom from CBPP has been maintained throughout 1996–2003 in Namibia (OIE, 2005a). The endemically infected northern reaches of the country have been kept separated by erecting a fence and maintaining sanitary regulations (Masiga *et al.*, 1996). Zambia has also devoted special attention to its border area with Angola (Beerling, 1991), but recent uncontrolled movement of people and their livestock across the border, resulting in the spread of CBPP in the northwestern and southern parts of the country.

Most of the cattle in Somalia are found in the central southern and northwestern parts of the country, but CBPP has been reported in both regions

(Mares, 1951; Mares, 1954; Edelsten, 1994; VSF–Switzerland, 2002; Hopkins, 2003).

2. 2. 3. Disease in Sudan

In Sudan, CBPP is considered the most serious disease of economic importance, which adversely affects foreign trade (Abdulla 1975, Shallali, El Mahi, Yazeed and Abdalla 1998). The disease was first observed in Darfur province in 1875 and later on, spread to Khartoum province where it caused great losses among cattle. Following a period of reduced incidences, the disease reappeared again during period of Mahadi revolution in 1889 (Anon, 1925). Thus it appeared in Kordofan province in 1912 and spread quickly southward and eastward. In 1913, it was reported in Nuba Mountains, White Nile, Blue Nile, Upper Nile and Bahr El Ghazal provinces (Anon, 1913). In 1914, the disease reached Khartoum again and spread to Kassala province in 1917 and Berber province in 1923. It was then considered that the northern regions of the Sudan were apparently free from the disease; while the south Sudan was endemically infected (Jones. B, 2002). Recently, the disease was reported to be endemic in western, southern and central states of the country. In the northern River Nile and Northern state and eastern states (Kassala and Red Sea) the disease has not been reported for more than 20 years (Shalalli *et al*., 2008)

2. 3. Aetiology

The disease is caused by the organism now classified as *Mycoplasma mycoides* strain PG-1 (Edward and Freundt; 1956). Later *M.m* strain was classified into small colony SC and large colony LC types on the basis of growth and biochemical characteristics (Cottew and Yeast; 1978). The *Mmm*SC type, the causative agent of CBPP formerly called pleuropneumonia-like or-

ganisms (PPLO), belongs to Class: *Mollicutes*, Order: *Mycoplasmatales*, Family: *Mycoplasmataceae* and Genus: *Mycoplasma* (Edward and Freundt; 1969). *Mmm*SC is a member of *M. mycoides* cluster (*Mm subsp. mycoides* SC variant, *Mmm* LC variant, *Mm subsp. Capri*, *Mm subsp. Capricolum*, *Mm subsp. Capripneumoniae* and *M*. bovine group 7). The six *Mycoplasmas* share serological and genetic characteristics, and this causes taxonomic and diagnostic problems (Cottew, Beard, Damassa *et al*; 1987).

There are no internal membrane structures and no cell wall external to the plasma membrane. However, many strains possess surface structures equivalent to a capsule.

2. 4. Epidemiology

Mycoplasma mycoides subspecies mycoides SC type, the causative agent of contagious bovine pleuropneumonia (CBPP), can be categorized into two major, epidemiologically distinct clusters: Cluster one contains strains isolated from different European countries since 1980 and Cluster two contains African and Australian strains collected over the last 50 years (Vilei *et al*, 2000).

In Africa the epidemiology of CBPP is dominated by different factors. These include the singularity of cattle as the only species affected, lack of reservoir host in wild the predominance of direct contact of susceptible animals with clinical cases or chronic carriers as the means of transmission and uncontrolled movements of cattle, all of which play a very important role in the maintenance and extension of the disease (Bessin and Connor, 2000).

2. 4. 1. Host range

Cattle of all types both (*Bos taurus* and *Bos indicus*) are susceptible. Domestic buffaloes are generally more resistant. However, CBPP has been reported

in Asian yaks (*Poephagus grunnien*, formerly *Bosgrunnien*) and in American bison (*Bison bison*) but never in African buffaloes *(Syncerus caffer)*. Sheep and goats are resistant to the disease (Provost, 1988).

There are variations in breed susceptibility in cattle; for example, trypanotolerant breeds seem to be more susceptible. Wildlife bovidae may also act as a reservoir for the causative agent (Yediontsclnig and Dardiri; 1976). Small ruminants may act as passive carriers of the disease when experimentally infected (Dick; 1937 and El Mahi; 1980). Young calves (less than one year of age) do not play the role of being reservoirs of infection because they show the infection in the form of an arthritis, rather than respiratory form (Multon *et al.*, 1956, Harbi and Salih; 1979, and El Mahi; 1980).

2. 4. 2. Sources of infection

*Mmm*SC occur in great numbers in bronchial secretions, nasal discharges, exhaled air and nasal aerosols. Spread of infection through urine droplets has not been fully confirmed. Microorganisms have also been isolated from bull semen, but transmission through semen requires further investigation.

2. 4. 3. Transmission

The disease is transmitted almost by direct contact between infected and susceptible cattle, by means of infected aerosols which may be carried over a distant of 200 meters by the air currents (Turner; 1959, FAO; 2003). Factors which influence the rate of spread of the disease are conditions under which cattle are herded closely together, intensity of infection and level of individual susceptibility (Turner; 1954). According to Masiga *et al.*, (1972), Scundamore (1976) and El Mahi (1980) *Mm* was isolated from the urine of infected animals and this contaminate the pasture and so causes indirect spread of the disease. Typical pulmonary lesions of pleurapneumonia were reported

in five (5) out of six (6) cattle fed fodder infected with virulent culture of *M. mycoides* (Windsor and Masiga; 1977). The organism was re-isolated from all infected animals. *Mm* can pass through the placenta from the infected dams to the off-spring (Stone; 1969).

The disease is not transmitted through contact with excreta, animal housing and equipment or vehicles previously occupied by infected animals (Turner, 1954; Hudson, 1971; Newton and Norris, 2000; Thiaucourt *et al.*, 2004a). In addition, cattle products (beef, milk and hides) are highly unlikely to transmit CBPP.

2. 4. 4. Incubation period

Experimental reproduction of CBPP is difficult but best achieved by bronchial intubation of low passage of *Mmm* as reported by (Gourlay and Howard; 1982, Martel *et al.*, 1983). In general incubation period ranges between 3-6 weeks and may reach up to 3 months (Thiaucourt *et al.* (2004a). They also maintained that *Mmm*SC 'can be present in the nasal passages of cattle for 40 days during the incubation period of the disease, before any serological response can be detected', and that this phenomenon may play an important role in the spread of the disease.

2. 4. 5. Morbidity and Mortality

Levels of morbidity for CBPP vary enormously in different situations and help to explain the epidemiology of the disease (Hudson, 1968). When newly introduced into naive cattle populations (those not previously exposed to the disease), morbidity may be close to 100% and mortality close to 50% (Newton and Norris, 2000). However, mortality rates in Africa typically range between 10-70% in epizootics characterized by low morbidity and low or nonexistent mortality, with the majority of infected animals showing

chronic lesions (Masiga et *al;* 1996; Regalla; 1996), This difference may be due to the fact that the European breeds (cattle) are generally in good health, better fed, and less subjected to physical stress (Nicholas and Palmer; 1994) and probably subjected to lower strains of *Mm* than in Africa (Abdo *et al;* 1998). In groups of susceptible cattle the morbidity approaches 90%; the case mortality may be as high as 50% and 25% of the infected ones remain as recovered carriers with or without clinical signs (Radostits *et al*., 1994).

2. 4. 6. Role of the carrier animals (lungers)

These are apparently healthy animals that have a localized focus of infection sequestered in a fibrous capsule in their lungs. Such animals are often referred to as "lungers". The organism can persist in such lesions for many months, and in time the fibrous capsule may break down, allowing viable organisms to escape by the bronchi and so infect susceptible in-contact animals. This is particularly prone to occur when chronic carrier animals are subjected to stress, such as when mustered or walked for long distances. Carrier animals are dangerous because they frequently introduce the disease into previously uninfected areas. In fact most new outbreaks had been traced to lunger animals or healthy looking animals as the source of infection. Newton and Norris (2000) maintain that sequestra were 'the source of many new outbreaks of disease' and that silent transmission occurred over distances of 1600 km in Australia.

2. 4. 7. Outbreaks

Outbreaks are triggered usually by the introduction of infected cattle into a naïve herd. Recovered cattle harboring the infectious organisms (lungers) could also become the source of outbreaks when such silent carriers are sub-

jected to stress, although this has not been substantiated experimentally (Windsor and Masiga, 1977).

In Africa, Asia, Eastern Europe, and the Iberian Peninsula, CBPP is still an endemic disease (Radostits *et al*., 1994). Because of the method of spread, outbreaks tend to be more extensive in housed animals and in those on transit by train or on foot and the incubation period can last from a few days up to several months.

2. 4. 8. Resistance

Up to one third of cases in Africa that recover from acute CBPP become potential carriers, probably this figure is higher in Europe where there is more widespread use of antimicrobial (Nicholas and Palmer, 1994). The use of antibiotics and anti-inflammatory drugs may help to mask clinical signs and accelerate the formation of chronic lesions.

Epidemiological and clinical observations indicate that the European outbreaks of CBPP are less virulent than the disease encountered in Africa. Furthermore, CBPP in Europe seems to be far more insidious, as it is usually chronic, and there are few distinctive clinical signs among affected cattle and rarely die (Vilei *et al*, 2000).

2. 5. Pathogenesis

CBPP is one of the multi-factorial diseases, in which the predisposing factors to the disease include: inter current infections, crowding, bad climatic conditions, age, genetic constitution, and stress from transportation, handling, and experimentation, all of which are important determinants of the final outcome of infection (Rosendal, 1993). The pathogenesis of the disease starts by thrombosis in the pulmonary vessels, probably prior to the development of pneumonic lesions. The mechanism of development of the throm-

bosis is not well understood, but is considered, at least in part, mediated through induction of cytokines (Rosendal, 1993).

Contagious bovine pleuropneumonia is presented as a variety of pneumonias in which the inter-lobular septa are extended and prominent due to a great out flowing of plasma and fibrin, giving the result of “marbling appearance” effect to the lung in these areas (Radostits *et al*., 1994).

Bronchitis, bronchiolitis, and alveolitis with predominantly neutrophils and mononuclear cellular response constitute the very early inflammation in *Mycoplasma* pneumonia. CBPP is characterized by substantial unilateral pulmonary necrosis, sometimes sequestration, and marked serosanguinous fluid accumulation in interstitial and pleura (FAO, 1997). Vasculitis appears to be an important component of the pathological changes in this disease, explaining the marked exudation and pleurisy. Thrombosis can explain ischemic necrosis and infarcts of the lung. Death results from anoxia and presumably from toxaemia (Walker, 1999).

2. 6. Clinical signs

The early stages of the disease are indistinguishable from any sever pneumonia with pleurisy (Scudamore, 1976). According to the report of Turner (1959), CBPP may be clinical or sub clinical. The clinical form may be acute, sub acute or chronic. Sub clinical and chronic forms of the disease constitute more than 50% of animals involved in the epizootic (Bygrave *et al*, 1968). Arthritis characterizes the primary picture of CBPP in calves of one year old or less, in which the pulmonary cavity may be free from any lesions (Moulton *et al;* 1965; Turner and Trethewie; 1961, Simmons and Johnson, 1963; Harbi and Salih, 1979; and Elmahi, 1980).

There is considerable variation in severity of symptoms observed in cattle affected by CBPP, ranging from hyperacute through acute to chronic and sub-clinical forms (European Commission Health & Consumer Protection Directorate General, 2001) and summarized as follows.

2. 6. 1. Per acute forms

The clinical signs observed in per acute form are much accelerated. Affected animals may die within a week exhibiting classical respiratory signs. In fatal cases, death occurs after a variable course of from several days to 3 weeks. The pathological signs are usually characteristic by marked pleural adhesion accompanied by exudative pericarditis and myocarditis (Provost *et al.;* 1987).

2. 6. 2. Acute forms

Animals show dullness, anorexia, drop in milk production, irregular rumination with moderate fever and signs of respiratory distress. Coughing is usually persistent and is slight or dry. Sometimes fever goes up to 40 – 42 °C, and the animal prostrates with difficulty of movement. Complication in calves may include valvular endocarditis and myocarditis (Martel *et al.;* 1983).

As the typical lung lesions develop, the signs become more pronounced with increased frequency of coughing and the animal becomes prostrate or stands facing the wind with the back arched, neck extended and elbows abducted, abdominal with painful grunting and dilation of the nostrils with excess mucous and frothy saliva around the mouth (Bygrave *et al.;* 1968 and Hudson., 1971).

While classical respiratory signs may be evident in calves, articular localization of the causative agent with attendant arthritis usually predominates. The clinical picture of infection in many calves is manifested by arthritis (Turner,

1959); the chest may be free from lesions even though the causative agent is introduced in the pulmonary system (Harbi and Salih, 1979, and El Mahi, 1980).

2. 6. 3. Sub acute forms

Signs may be limited to a slight cough only noticeable when the animal is exercised. Cattle that recover naturally are extremely weak and emaciated. The lesions are localized in small part of the lung and the position cannot be easily detected by percussion and auscultation. New foci of infection are created sometimes (Henning, 1956).

2. 6. 4. Chronic forms

In the chronic stage of infection, no infected droplets are detectable in expired air, even following coughing, and it is presumed that animals in this stage of the disease are much less infectious than those in the acute phase (Newton and Norris, 2000).Many infected animals develop chronic or milder forms of the disease, which may be either symptomless or associated with only a slight temporary rise in body temperature, and some loss of condition. Recovered animals may be clinically normal but in some, an inactive sequestrum forms in the lung, with a necrotic centre of sufficient size to produce a toxaemia causing unthriftness, a chronic cough, and mild respiratory distress on exercise.

2. 7. Pathology

The characteristic postmortem findings in CBPP are localized in the thoracic cavity and lungs, except in young calves where inflammation of the limb joints (usually the carpal and tarsal joints), with increased fluid, is sometimes seen. There is thickening and inflammation of the pleura often with heavy deposits of fibrin and large amounts of clear, serous effusion contain-

ing shreds of fibrin. A most striking feature of the acute disease is the very large volume of yellow fluid (up to 30 liters) containing clots, which can accumulate in the chest and therefore causing extremely difficult breathing (FAO, 1997). This pleural fluid is the ideal diagnostic material from which the causative *mycoplasma* can be isolated from or on which PCR can be carried out with DNA purification (Nicholas and Bashiruddin, 1995).

The lungs (almost always only one, the left) and pleura are affected and in most cases, only the diaphragmatic lobe is affected than the cranial lobe (Nunes and Pestica *et al.;* 1988; 1990). Affected lobules show various stages of gray and red hepatization and the interlobular septa of the affected lungs are greatly distended with serofibrinous exudates, the classical 'marbled' lung of this disease (Radostits *et al.*, 1994). In acute forms, the yellowish fluid in the thoracic cavity may solidify and cover the lining of the thorax and surface of the lung (the pleura) with a yellow or yellowish-grey fibrin coating resembling an omelet. Accumulation of fibrin on the pleura causes the lung and chest wall to stick together (adhesion). In the recovered and chronic form, fluid is rarely seen in the pleural cavity but adhesions between lung lobes and between lungs and the chest wall are commonly found. Infarcts, varying in size from about 10-300 mm, are frequently preset in the affected lung tissue, which are the result from thrombosis of inter- or intralobular arteries and lymph vessels. The infarcts subsequently become sequestered from the adjacent parenchyma by granulation tissue/fibrous capsule. The diameter of a sequestrum can vary from 2 to 25 cm and the capsule can be as much as 1 cm thick (FAO, 1997). The sequestrum may constitute a source of infection to cattle when ruptured, particularly where the sequestrum is drained by bronchus which forms an outlet for dissemination of in-

fected aerosols droplets (Provost *et al.;* 1987), such mechanism may account for outbreaks of disease in closed herds (Windsor and Masiga; 1977).

The mediastinal and bronchial lymph nodes are enlarged and wet (edematous), with small necrotic foci and pinpoint hemorrhages, on section the inner surface is either normal (yellowish) congested or granular (Bygrave *et al.;* 1968). In the kidney sharply demarcated renal infract are clearly visible when the capsule striped (Hudson, 1971, and El Mahi, 1980).

In the heart especially in calves, the lesions were described as vegetative vulvitis, endocarditis and myocarditis (Turner and Trethewie, 1961).

In the calves, the lesions are often restricted to the joint and bursa (Moulton *et al.;* 1956; Turner and Trethewie 1961; Simmon and Johnson, 1963; Harbi and Salih, 1979 and El Mahi, 1980). According to Turner and Trethewie (1961) the lesions in the joints and bursae are non purulent serofibinous polyarthritis characterized by hyperemia and effusion. Affected calves may have exudative peritonitis, arthritis, bursitis and fibrinous arthritis of the carpal and tarsal joints (Provost *et al.;* 1987).

2. 8. Diagnosis

The diagnosis of all phases of CBPP on clinical ground is very difficult since the symptoms simulate those of other forms of pneumonia (Turner and Hudson, 1971). The diagnosis is based on a history of contact with infected animals, clinical findings, immuno-diagnosis tests, necropsy findings and cultural examination. The most sensitive diagnositic tests are:

1. Competitive Enzyme Linked Immuno Sorbant Assay (c-ELISA)
2. Complement Fixation Test (CFT).

2. 10. Treatment

The most common antibiotics livestock keepers used for minimizing CBPP cases at field level are Oxytetracyclines, Tylosine, erythromycin, lincomycin, spectinomycin and tilmicosin (Walker, 1999). Tylosin and spiramycin are effective in control of excessive vaccination reactions and should be of value in the treatment of clinical CBPP (Radostits *et al*, 1994). The problem of using antibiotics is that it leads to development of carrier animals (Lungers).

2.11. Control and eradication

Testing of individual animals, slaughter of all reactors, with compensation of the cattle keepers (stamping out), and vaccination of the non reactors by reliable immunizing product and practice of stringent monitoring methods are the most effective method of eradication of CBPP. This can only be carried out at a very initial expense (Abdulla; 1969).

CHAPTER THREE

MATERIALS AND METHODS

3. 1. Description of study area

Western Bahr El Ghazal State is one of the 10 States in Republic of South Sudan. Formerly it was known as the Greater Bahr El Ghazal Region and comprised the current four states namely Western Bahr El Ghazal State (WBGS) Northern Bahr El Ghazal State (NBGS), Warrap State (WS) and Lakes State (LS).

Western Bahr El Ghazal State covers a total area of 91,902 sq km. It is located in the north-western part of Southern Sudan and lies between latitudes 6.31 degrees and 10.29 degrees North of the Equator, and between longitude 23.59 degrees and 28.22 degrees East of the meridian.

Western Bahr El Ghazal State shares international borders with Central Africa Republic (CAR) to the south west, Republic of Sudan to the west, and in Southern Sudan it borders Western Equatoria State to the south, Northern Bahr El Ghazal to the north and Warrap State to the east.

The vegetation is broad-leafed woodland, with several varieties of trees. The State is naturally rich with various plant species, however, Lulu trees; Mahogany, Yubu and Palum Surge cover a large area. In addition to the man-made forest, including limited species of trees such as Teak, Eucalyptus, Acacias and Neem trees were planted in the state.

Western Bahr El Ghaza State has 7 to 9 months crop season dominated by wet monsoon climate and, medium wet season. Moreover, the State is also affected by between 5 to 7 months of humid weather and a dry spell of 3 to 5 months *(Isaac Bior and James Odra, (Eds), 2002, P. 31).* The rainfall starts

from April and ends in October and sometimes extends up to November. The main annual rainfall is estimated between 900 – 1,300 mm per annum. The rains are favorable without adverse effects which is good for crop productions *(Special Report, WFP/FAO, Feb 2009).*The average maximum temperatures during the hottest months range between 34-39^{0}C and the average minimum temperatures during the coldest months are between 10-12^{0}C.

Western Bahr el Ghazal is a multi-ethnic, multi-cultural and multi-lingual State. It is inhabited broadly by three major groups: the Fertit which are the majority, Loa (Jur Chol) and Dinka Marial Bai group. The Dinka Marial Bai and Jur Chol groups are agro-pastoralists compared to Fertit group that depend entirely on cultivation of crops, but some of them have recently started to keep cattle.

The total population of Western Bahr El Ghazal State is estimated at 333, 431people (*the fifth Sudan population and housing census, 2008*).

The livestock possessed by the communities in Western Bahr El Ghazal State are cattle, estimated as 1,200,000 head, goats 850,000 and sheep 600,000 head (*Rinder pest vaccination, 1997*). This is not including the livestock of the nomadic Follany (Falata) tribes and Arab tribes that come from Republic of Sudan and Central Africa Republic (CAR) for pastures and water during the dry season.

The cattle breed in the area is predominantly the *Nilotic* breed, which is known for a relatively small size (250 – 300 Kg for adults) and big horns, with many different coat colors (see Figure 2).

3. 2. Study design

A Cross-sectional survey was carried out in two counties of Western Bahr El Ghazal state (Wau and Jur River County). Eleven (11) villages were purpo-

sively selected based on cattle population and CBPP status. Animals were examined clinically. Participatory Disease Search techniques (semi-structured interview, proportional piling and seasonal calendar) were applied to analyze community based approaches to CBPP and other diseases prevalent in the area. Geographical position system (GPS) was applied to label the spatial distribution of diseases from participatory epidemiological analysis and serological survey.

3. 3. Sampling frame and sampling size determination

The sampling frame consisted of list of villages and associated cattle population in these Areas. The population of cattle in each village could not be obtained except the list of the villages. The sampling method used was purposive sampling based on two categorizes or groups. Group (A) were animals that had been infected with CBPP for the last 2-3 years, and group (B) were animals that were infected with CBPP less than 1 year or had clinical signs of CBPP.

Since the prevalence of CBPP in Western Bahr El Ghazal state was not known, 20% prevalence was considered. From each herd 20% of animals were selected using random sampling procedure (Thrusfield 1995).

Out of 700,000 head of cattle in Jur River County 168 animals were sampled from 8 different sites, while in Wau County 123 animals were sampled out of 400,000 head in 4 different sites.

In this investigation a total of 291 serum samples were tested from two groups of cattle (143 from group A, and 148 from group B); group A are cattle that have been infected with CBPP for last 2-3 years, and group B are cattle that were infected with CBPP less than 1 year or have clinical sings of

CBPP. These sera were collected from 43 cattle herds, 5 payams and two counties of Western Bahr El Ghazal State.

The risk factors were identified based on the retrospective data from semi-structured interview with the key informant groups who were selected from the cattle – keeping communities during Participatory Disease Search.

3. 4. Participatory Disease Search (PDS)

PDS exercise was conducted in two counties of Western Bahr El Ghazal state (Jur River and Wau Counties), covering 11 different villages/herds. The methods used in this research were semi-structured interview (SSI), focus group discussions, open ended questions, and probing questions, in addition to proportional piling and seasonal calendar. Participatory disease search (PDS) was used in this study with the objective of finding carrier animals (lungers), which could have been responsible for clinical CBPP in the study area.

3. 4. 1. Proportional Piling

The five most important cattle diseases were selected by the informant groups and they were asked to rank the diseases according to their impact using 100 piles of stones against 5 diseases.

3. 4. 2. Seasonal Calendar

Here the respondents were asked to mention the seasons of the year in their areas using local names for seasons and each season was represented by an object. Stick with big green leaf represented *Keer/Ruowl* (June-October/rainy season), followed by smaller dry stick *Apuok pai* (Nov.-December/beginning of dry season), stick with small green leaf *Yakthok* (April-May/beginning of rainy season) and bigger dry stick *Mayi* (January-

March/dry season). The respondents were requested to explain the meaning of each symbol to know whether they have understood what it represented. The respondents were given 30 stones and asked to show the relative occurrence of each disease in each season.

3. 5. Collection of serum samples

Animals were restrained by owners and 10 ml of blood sample were collected from the jugular vein, using vacutainer tubes. The samples were kept for four hours and centrifuged. The sera were transferred to serum tubes and kept at - 20 ^{0}C until brought to the laboratory for analysis. Corresponding to each sample, the sex, age and group or category of every animal and geo reference information were collected and put on a separate note book.

3. 6. Clinical examination

Clinical examinations of sick animals due to CBPP were done in order to cross-check and triangulate the perception of the livestock keeper with other participatory appraisal results. Thirty four animals which showed respiratory signs were clinically examined by the research team. The cattle keepers identified the suspect animals for clinical examination. Clinical signs considered were anorexia, dyspnoea, arch back and abduction of forelimbs, exercise intolerance, cough, nasal discharges and lagging behind the group during movement.

3. 7. Serological testing

3. 7. 1. Competitive ELISA test

A competitive enzyme-linked immunosorbent assay (c-ELISA) developed by the OIE Collaborating Centre for the diagnosis of animal diseases in tropical countries was applied. The c-ELISA is now provided as a ready made kit that contains monowell coated micro plates, which are washed with con-

centrate (20x), dilution buffer (24), controls: strong positive control (CP++), weak positive control (CP+) and negative control (CN), monoclonal antibody 117/5 (anti-MmmSC)-(Mab)-(Freeze-dried), Monoclonal anti-mouse IgG peroxidase conjugate, Revelation solution (3)-tetramethyl benzidine (TMB) ready to use and stop solution (H2S040.5M solution).

3. 7. 2. Equipment

Micro-plate washing system that distribute 300 microleter per well, tray, precision micropipettes and multi-dispensing micropipettes, disposable pipette tips, distilled water, pre-plates for pre-dilution, microplates covers, incubator at +37°C, plate agigator and printer.

3. 7. 3. Method

The wells of the polystyrene microtiter plates were coated with an *Mmm*SC lysate. Serum samples to be tested were diluted and incubated with the specific monoclonal antibody (117/5) in a pre-plate. This mixture was then transferred into the *Mmm*SC coated micro plate. Any antibody specific to *Mmm*SC in the serum will form an *Mmm*SC/bovine antibody immune complex, which effectively masks the *Mmm*SC site. In this case the monoclonal antibody cannot bind to the corresponding epitope.

After washing, anti-mouse-IgG antibody coupled to peroxidase was incubated in the wells. The presence of specific *Mmm*SC antibodies in the serum that is being analyzed, the monoclonal antibody (117/5) is not fixed in the plate and the conjugate cannot bind in the wells. On the contrary, the conjugate can bind to the monoclonal antibody.

After washing, the enzyme substrate (TMB) was added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of

color is an inverse measure of the proportion of anti-*Mmm*SC antibodies in the serum sample tested.

The cut off was calculated by using the results obtained from a monoclonal control (Cm) and conjugate control (Cc). The positive and negative controls were delivered with the kit. They were added to each micro plate and the results were validated

3. 7. 3. Competitive ELISA test procedure

1. Dilution buffer (100 µl) was dispensed in the wells of pre-plates
2. 11 µl of the 3 control samples were dispensed: CP++ in B1, B2, C1, C2 and CP+ in D1, D2, E1, E2 and CN in H1, H2 follow by the dispensing of 11 µl of "Dilution buffer" in the wells A1, A2.
3. 11 µl of serum was added in the other wells (A3 to H12), 110 µl of diluted monoclonal antibody (MaB) was added except wells A1 and A2 which are hereafter called conjugate control (Cc).
4. The mixture was transferred from pre-plates to coated plates and incubated for 1 hour at 37°C.
5. The plates were washed twice and conjugate is added to all the wells (100 µl); the wells were then incubated for 30 minutes at 37°C.
6. The plates were washed three times and the substrate was added to all the wells (100 µl), and the plates were incubated for 30 minutes at 37°C. Then reading was performed by computer.

CHAPTER FOUR

RESULTS

Based on the serological, clinical and participatory epidemiology (PE) techniques, CBPP was found to be the major cattle health problem in Wau and Jur River counties in Western Bahr El Ghazal State.

99% of tested cattle were Nilotic cattle breed, with big horns. The majority of samples (98%) were collected from Dinka cattle, while 2% were collected from Jur Chol and Fertit cattle. In this research 96% of the sampled animals have been vaccinated against CBPP together with BQ, Anthrax and HS. Vaccination is a routine programme the State Ministry of Animal Resources and fisheries organizes annually.

4.1. Prevalence of Contagious Bovine Pleuropneumonia (CBPP) using c-ELISA

During this study a total of 43 herds were surveyed and 291 sera were tested from 2 Counties in Western Bahr El Ghazal State. Out of the tested sera by c-ELISA, 13% showed positive reactions, 68% showed negative reactions and 18.9% showed doubtful reactions as mentioned in table (1).

Table 1: Prevalence of CBPP in Western Bahr El Ghazal State (Jur River and Wau counties combined) by c-ELISA.

State	*c-ELISA results*				*Total*
	No. of-tested herd	*Positive*	*Negative*	*Doubtful*	
Western Bahr El Ghazal	43	38	198	55	291
%		13%	68%	18.9%	100%

Table 2: Prevalence of CBPP in the two study Counties, Bahr El Ghazal State by c-ELISA

County	*c-ELISA Result*				*Total*
	No. of tested herd	*Positive*	*Negative*	*Doubtful*	
Wau	14	19	77	27	123
%		6.5%	26.4%	9.2%	42.1%
Jur River	29	19	121	28	168
%		6.5%	41.5%	9.6%	57.6%
Total	43	38	198	55	291

Table 3: Prevalence of CBPP based on two categories of cattle (Groups A & B), for both study counties of Bahr El Ghazal State/c-ELISA test.

Category		*c-ELISA results*			*Total*
		Positive	*Negative*	*Doubtful*	
Group A		19	95	27	143
	%	6.5%	32.6%	9.2%	39.1%
Group B		19	103	28	148
	%	6.5%	35.3%	9.6%	41.8%
Total		38	198	55	291

Table 4: Prevalence of CBPP based on sex in both study counties.

Sex of animal		*c-ELISA results*			*Total*
		Positive	*Negative*	*Doubtful*	
Male		13	67	15	95
	%	4.4%	23%	5.1%	32.5%
Female		25	131	40	196
	%	8.6%	45%	13.7%	67.3%
Total		38	198	55	291

Table 5: Prevalence of CBPP based on age in both study counties.

Age of animal		*c-ELISA results*			*Total*
		Positive	*Negative*	*Doubtful*	
Adult		36	161	46	243
	%	12.3%	55.3%	15.8%	83.4%
Calf		2	37	9	48
	%	0.9%	12.7%	3.1%	16.5%
Total		38	198	55	291

Table 6: CBPP sero-prevalence by Location, (both study counties).

Locations of samples		*c-ELISA results*			*Total*
		Positive	*Negative*	*Doubtful*	
Majak		7	46	17	70
	%	2.4%	15.8%	5.8%	24%
Factories		4	9	8	21
	%	1.3%	3%	2.7%	7%
Eastern bank		3	19	6	28
	%	1%	6.5%	2%	9.5%
Wount Ngot		3	14	2	19
	%	1%	4.8%	0.7%	6.5%
Mangang	%	0	7	2	9
		0	2.4%	0.7%	3.1%
Tharqueng		1	11	2	14
	%	0.3%	3.7%	0.7%	4.7%
Tharqueng		2	9	2	13
river	%	0.7%	3%	0.7%	%
Ngojur		8	22	2	32
	%	2.7%	7.5%	0.7%	9.9%
Riny Aleg		3	23	8	34
	%	1%	7.9%	2.7%	11.6%
Marial Ajith		5	21	2	28
	%	1.7%	7.2%	0.7%	10.6%
Zogolona		2	17	4	23
	%	0.7%	5.8%	1.3%	7.8%
Total		38	198	55	291

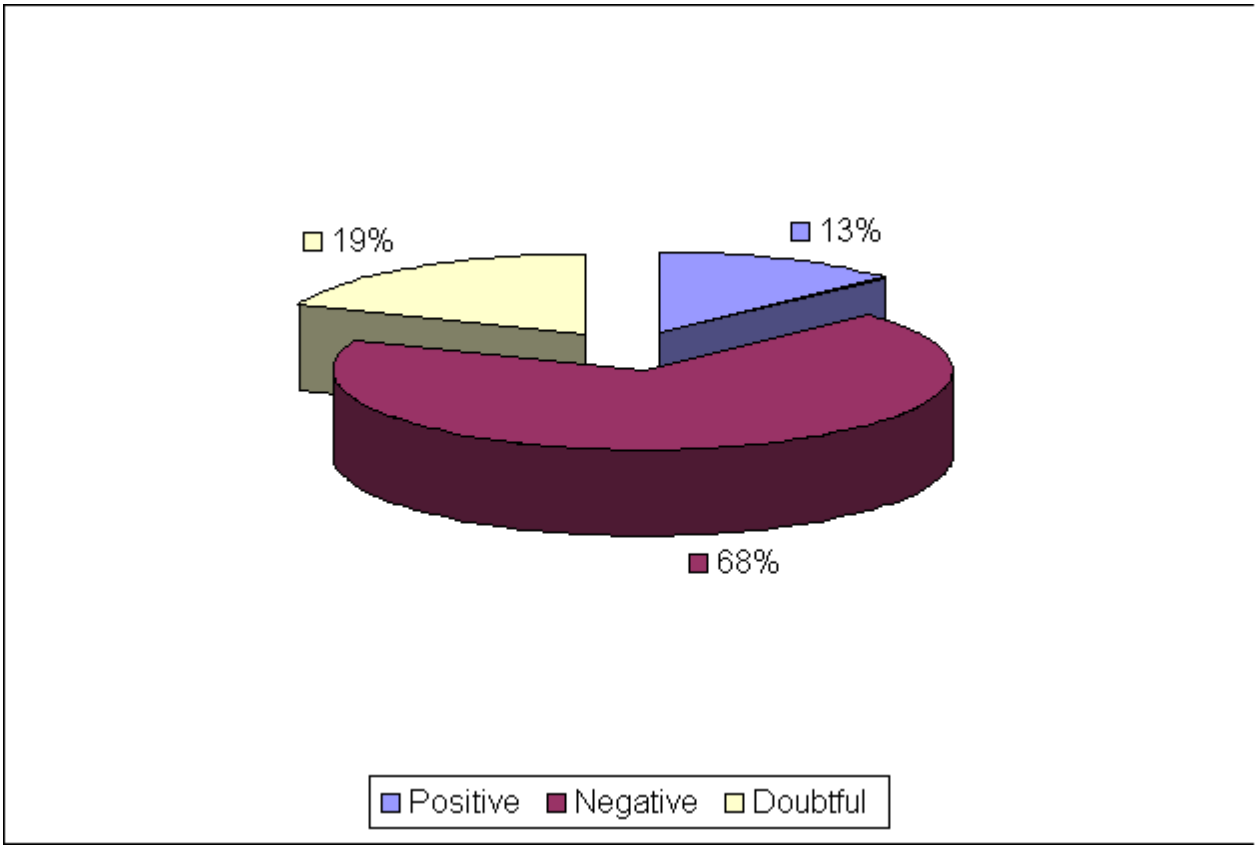

Figure (1) Prevalence of CBPP in the study area.

Figure 2: The neck extended, mouth opened with froth, facing wind, difficulty in breathing and abduction of forelimbs in this cow with CBPP

Figure 3: Four (4) hours later. Prolong recumbence with extended neck, opened mouth with froths in the same cow with CBPP.

Figure 4: Arch back, abduction of forelimbs and difficulty in breathing in calf with CBPP

Figure 5: Extended neck, arch back and abduction of elbow in cow with CBPP

4. 2. Participatory Disease Search (PDS)

In this exercise participatory methods were used to understand the local perceptions of livestock keepers with respect to animal health in general and CBPP in particular in the study area. The methods used were semi-structured interviews (SSI), focused on group discussions, proportional piling, seasonal calendar, observation and transit movements. In addition, participatory enquiries using both open - ended and non - directed questions such as (who, why, where, when, what, how) and probing questions (which were directed to CBPP) were used.

Moreover, the methods of CBPP control were asked by the researcher team and found out that pastoralists were using various types of antibiotics (Tetracycline, Tylosin…etc) to minimize CBPP cases. And they have unanimously agreed that the use of antibiotic treatment was the best choice they have got, since the veterinary services were very poor in some of the areas.

Table 7: CBPP outbreaks mentioned by the respondents in different locations during PDS interviews.

Location of outbreak	Year	No. of affected animals	No. of dead animals	No. at risk
Majak	2010	30	7	700
Agouk	1991	23	9	100
Panameet	2002	50	21	900
Achongchong	2006	39	13	300
Mangang	2006	31`	9	700
Nyn Akok	2001	43	20	300
Kuajok	2001	33	8	250
Tonj	1998	25	16	200
Kurajina	2000	19	8	400
Marial Bai	1997	40	20	500
Gati	2001	25	11	100
Total		358	142	4450

Table 8: Lexicon of local diseases terms in different location of field study area of Participatory Disease Search (PDS)

Local name	*Scientific name*	*Traditional case definition*
Acum	Worms	Weakness, dry faeces, drop in production
Maliei/Liei	Trypanosomiasis	Emaciation, drop hair of the tail, anemia
Abuot Poeh	CBPP	Cough, arch back, difficulty in breathing, drop in production, open mouth and death
Achiak	Ticks	Biting fly causing skin injures, anaemia, transmit diseases
Joong Nhial	Anthrax	Sudden death, blood in black colour from natural orifices, bloat
Deet	FMD	Wounds on hooves, mouth, lameness, death
Matonton	Lumpy Skin Disease	Various size of nodules on skin, fever, drop in production
Morol	HS	Swelling of the neck and chest, death after 2 days
Macu	BQ	Swelling of the legs, painful, lameness and death after few days
Athwung	Brucellosis	Abortion, retain placenta, swelling of joints

4. 2. 1. Proportional piling

Table 9: Cross tabulation of 5 important diseases prevailing in the two Counties (Wau and Jur River) in Western Bahr El Ghazal State.

Location	Disease										
	Worms +foreign body	**Tryps + tsetse fly**	**CBPP**	**FMD**	**Anthrax**	**HS**	**BQ**	**Ticks**	**LSD**	**Bru-cello-sis**	**Total of stones**
Majak	5	58	32	0	0	0	0	5	0	0	100
Factories	3	0	33	10	36	0	0	18	0	0	100
Eastern bank	0	23	26	0	17	0	0	0	16	0	100
Wountngot	18	12	39	0	24	0	16	0	9	0	100
Mangang	0	13	36	0	27	0	18	0	0	0	100
Tharqueng	0	13	28	0	26	6	22	0	0	0	100
Tharqueng river	0	13	40	0	26	11	11	0	10	0	100
Ngojur	13	0	43	0	0	0	0	0	14	5	100
Riny Aleg	0	23	26	0	17	0	18	25	16	0	100
Marial Ajith	0	14	35	3	41	0	0	0	7	0	100
Zogolona	0	20	26	8	36	0	0	0	0	0	100
Total	**39**	**179**	**364**	**21**	**250**	**17**	**85**	**58**	**72**	**5**	**1100**
%	**3.5**	**16.2**	**33.1**	**1.9**	**22.7**	**1.5**	**7.7**	**5.2**	**6.5**	**0.5**	

Table 10: prevalence of 5 important cattle diseases ranked by communi-ties, using PDS in Western Bahr El Ghazal State

Disease	Percentage	Ranking
CBPP	33.1	1
Anthrax	22.7	2
Trypanosomosis	16.2	3
Black Quarter	7.7	4
Lumby Skin Disease	6.5	5

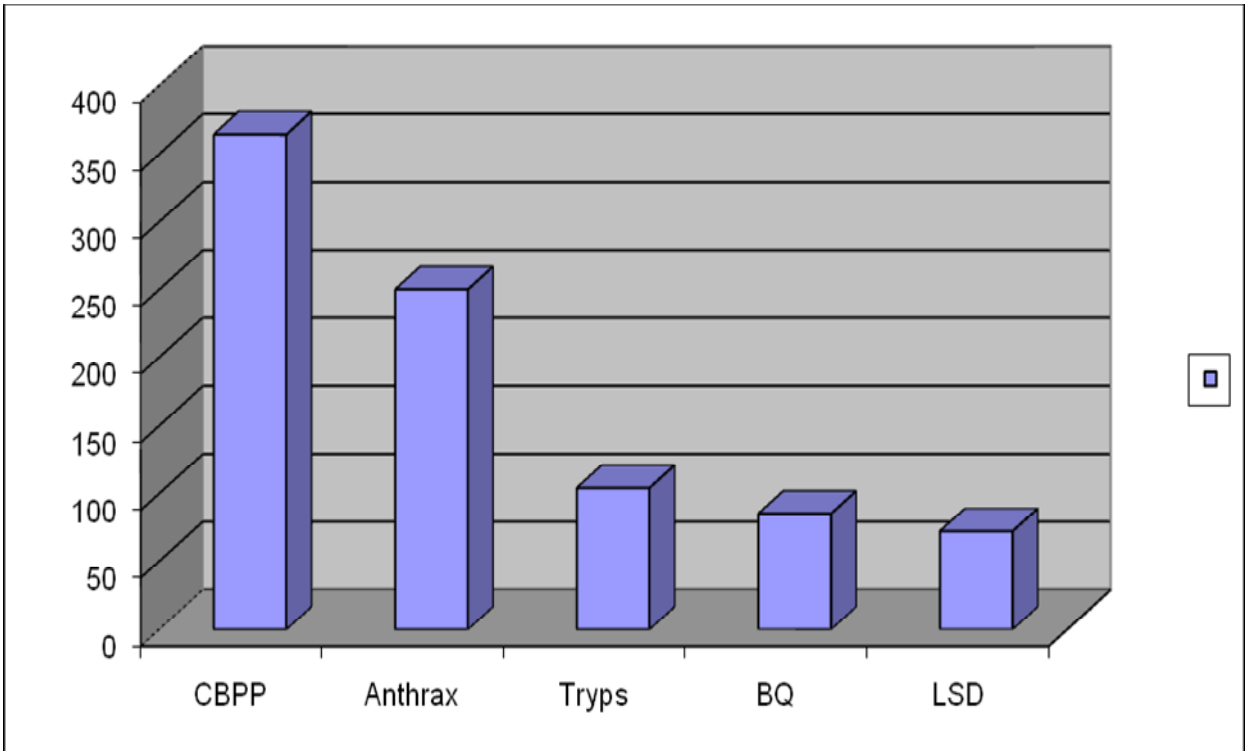

Figure 6: Proportional piling for the most 5 important cattle diseases ranked by the respondents in Western Bahr El Ghazal State.

4. 2. 2. Seasonal Calendar

Table 11: A summarized seasonal calendar of cattle diseases occurrence against 4 local seasons as mentioned by the respondents in the study area.

Diseases	**Seasons**			
	Mayi (Jan-March) Dry season	**Yakthok (April-May)** Beginning of rainy season	**Keer/Ruowl (June-October)** Rainy season	**Apuok Pai (Nov.-Dec)** Beginning of dry season
Anyour (for-eign body)	20			10
Deet (FMD)	15	66	6	3
Jong Nhial (Anthrax)	45	10		5
Liei/Maliei (Tryps)	107	67	18	71
Macu (BQ)	130			20
Adhowang (Brucellosis)	10	4	3	13
Matonton (LSD)		13	167	
Maaw (Tsetse fly)			30	
Acum (Worms)	42	12	10	26
Achiak (Ticks)		85	35	
Abuot Poeh (CBPP)		38	289	3

The figures 3 to 289 represent the number of stones that were used during the construction of the seasonal calendars.

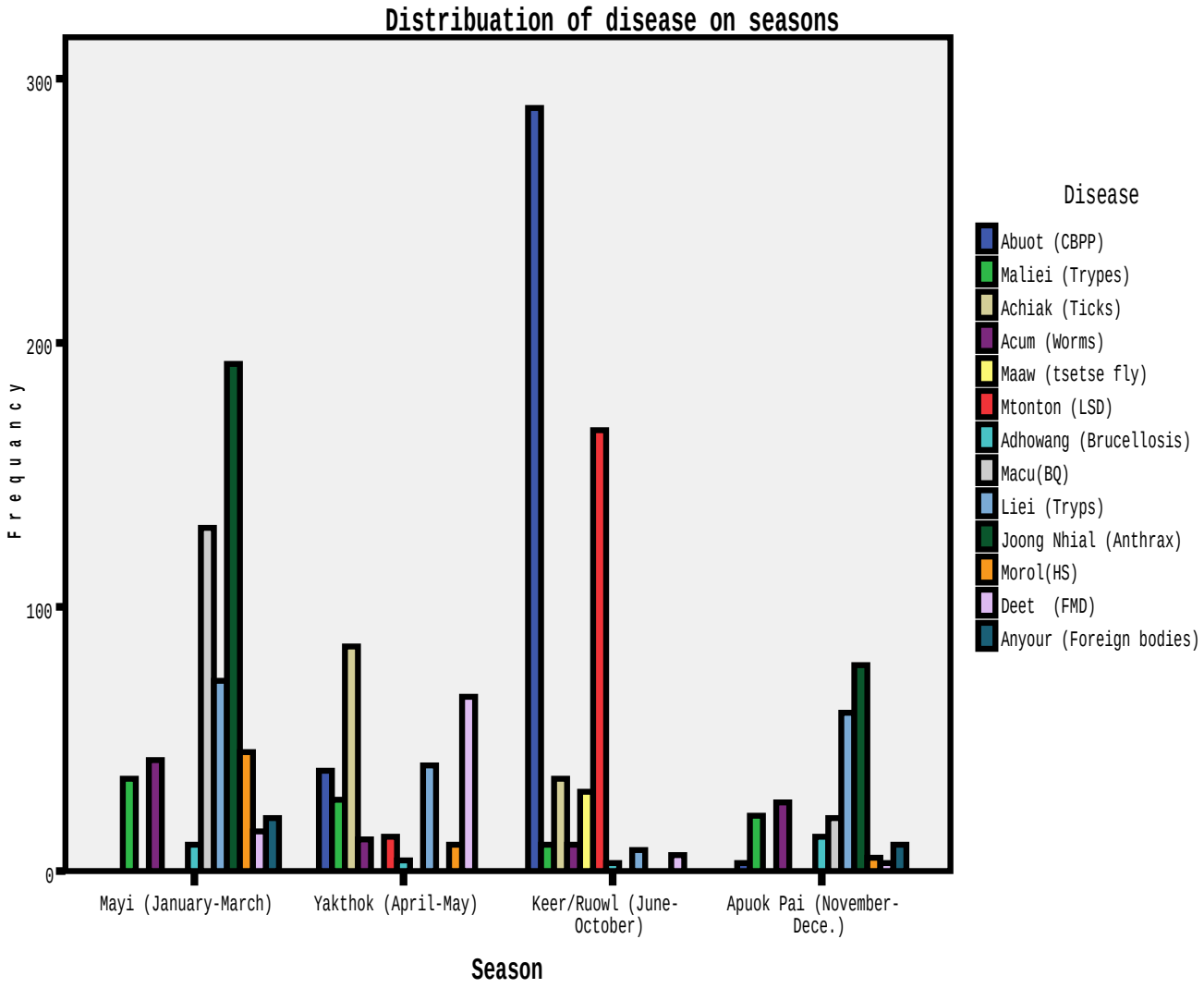

Figure 7: Distribution of the diseases on four seasons of the year as mentioned by the communities

CHAPTER FIVE

DISCUSSIONS

The local name given to CBPP by pastoralists refers to organ affected and its symptoms, such as *Abuot* mean *swelling* and *Poeh* infection of lung, all describe the disease. This indicates that the pastoralists have a thorough knowledge of the signs and affected organ. These results are compatible with those of *Zessin et al.* (1985), who suggested that a targeted approach to CBPP control would be warranted based on a disease intelligence system that involved the livestock keepers.

The results of competitive-ELISA in this study revealed that; (38) 13% of the tested sera were positive, (55) 18.9% were doubtful and (198) 68% were negative to c-ELISA. The positive result is compatible with the results obtained from PDS techniques which CBPP had got high mark among others diseases in most locations of interview. This indicated that the disease is common in the study area. Also in this study c-ELISA positive results revealed that 6.5% were for group (A) as well as group (B) and this is an indication that the disease is common among different ages of animals.

In this study, the c-ELISA suggested that 8.6% out of the positive results appeared in the females while only 4.4% appeared in the males, indicating that CBPP was more common among the females. This may be the females are always kept for propose of production rather than males that are always sent to the market for income.

The results of c-ELISA in this study revealed that; 12% out of the positive results appeared in the adult cattle and only 1% appeared in the calves, indicating that CBPP was very common among the adults. This may be the

calves still have antibodies against CBPP in their bodies or the random use of antibiotics by cattle keepers have reduced the morbidity rate.

The c-ELISA positive results illustrated in this research were also the same for Wau and Jur River County 6.5%. This indicates that the disease is endemic in both study counties.

The result of PDS using proportional piling showed that out of 5 important diseases prevailing in Western Bahr El Ghazal State, CBPP was first ranked as disease of priority in the area followed by Anthrax, Trypanosomosis, BQ and Lumby Skin Disease. And according to the results obtained from seasonal calendar (*Abuot Poeh*) CBPP was very common during wet season, this information could be a great value for CBPP surveillance and control strategies in the study area.

Also the respondents indicated that (*Abuot Poeh)* CBPP was endemic with peak of mortality during periods of stress such as the onset of rains. This result is consistent with Hudson's (1968); who reported that the organism is very sensitive to ultraviolet light and during the daylight in hot sunny weather droplets may soon be rendered harmless. Close contact at night and during dull weather may be important in the spread of infection.

The traditional exchange of livestock between the communities (Dinka communities) and husbandry system (e. g. smoke caused by burning of dry feces...etc to scare away fliese and other insects from cattle) would indeed appear to facilitate the establishment and maintenance of endemic stability of the disease.

Those results agree with the findings of Zessin *et al.,* (1985) who noted "Because Dinka husbandry system makes animals rotate and circulate extensively, leading to endemic 'disease stability'.

During the semi-structured interviews the respondents unanimously agreed that the use of antibiotics treatment for CBPP cases was best and the only option they have got in reducing mortalities due to CBPP. The same major conclusion was stated by Mariner, (2003) who mentioned that treatment is a much more commonly practiced intervention than vaccination. Also during the same interviews, the pastoralists mentioned the cattle that are coming from Warrap, Northern Bahr El Ghazal State, Republic of Sudan and Central Africa Republic for grazing during the dry season were the ones which contaminated their pastures with CBPP organism which facilitated establishment of endemic condition of the disease in the area. The same major conclusion was made by (Karib; 1958, Hudson; 1971 and Abdulla; 1975). These workers stated that in most African countries including Sudan (in particular south Sudan) where the disease is enzootic, the mode of animal husbandry is nomadic and restriction of movement is difficult; segregation, quarantine of infected herds, testing and removal of positive cattle and annual vaccination become the only routine methods for disease control. However, the uncontrolled animal movements during transhumance, trade, lack of checkpoints and quarantines, and cattle raiding have facilitated the spread of the disease throughout Western Bahr El Ghazal State.

These results are consistent with those reported by Msami *et al.*, 2001) for Tanzania where uncontrolled transhumance, trade and cattle theft have facilitated the spread of CBPP. Although the quarantine and checkpoints have been in place, weak legislation and a lack of means and resources to enforce control of livestock movements are making the situation worse.

The ideal method to control a trans-boundary disease like CBPP is the application of the stamping out policy (Hudson, 1971). This includes: complete

elimination of infected and exposed animals along with attendant zoo-sanitary measures through slaughter of all clinical cases and contact cattle, restriction of livestock movement in and around the infected area and payment of compensation to cattle owners. However, stamping out policy is the method of choice when the infection is detected early, the extension of outbreak is limited and adequate financial, infrastructural, and human resources to execute the task are ensured. But in cases where infection has progressed, involving large numbers of cattle, stamping out may not be the option of choice.

With regard to CBPP control, state ministry of animal resources and fisheries used to organize vaccination campaigns against CBPP with other bacterial vaccines every six months. In spite of the present vaccination interventions, the pastoralists still have the option of treating sick animals with antibiotics (Oxytetracycline 5%, 20% and Tylosine).

Eradication of CBPP is very difficult in south Sudan in general and Western Bahr El Ghazal State in particular due to free movement of nomadic tribes and lack of awareness among livestock communities about the good husbandry systems.

Botswana is a typical example of African country in eradicating CBPP recently from the country. This was in February 1995 after an initial freedom from the disease of almost half a century, the last outbreak having been reported from Chobe district in 1939. Since the disease has been absent for so many years, laboratory diagnostic capability for effective diagnosis of the disease was limited to gross and histo-pathological diagnosis and cultural isolation of the aetiological agent of CBPP. Therefore, the government of

Botswana and FAO carried out training of laboratory technicians and supervising officers on different serological techniques. (Amanfu, 2000a).

With the veterinary department's effort and assistance from FAO, the micro-method of Complement Fixation Test (CFT), which was OIE approved was established in the laboratory. In addition, the Serum Agglutination Slide Test (SAST) was likewise used as a pen-side test. Later, the Competitive Enzyme Linked Immuno-Sorbent Assay (C-ELISA) test and Polymerase Chain Reaction (PCR) were introduced as additional diagnostic test (Amanfu, 2000a). All these tools i.e. serology, isolation, clinical symptoms, pathology and epidemiology were used as effective means for decision making during the outbreak and for surveillance after the last suspected animal had been destroyed.

It must be pointed out that the commitment of government to get rid of CBPP and maintain freedom from the disease thereafter was responsible for the success that was achieved. It is also due to the role that cattle play in the socio-economic activities of the people of Botswana which accounts for 46 % of the GDP (Amanfu, 2000a). Generally, the major feature of Botswana success in CBPP eradication are; availability of equipment and reagents, veterinary staff and farmer training, quality assurance, proficiency testing and considerable efforts in achieving the desired results.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The participatory epidemiology (PE) results were found to be consistent with the conventional veterinary methods of diagnosis of CBPP and were found to be the best approach in pastoral community for gathering disease intelligence.

The major risk factors identified during this study were the types of husbandry system and associated cattle movement. Moreover, CBPP was found to be endemic in the study counties of Western Bahr El Ghazal State. Uncontrolled movement of animals, cattle raiding, use of antibiotics, which facilitate development of carrier animals (lungers), all constitute a major problem in the study area. The study confirmed that the carrier animals (lungers) were found to be responsible for clinical CBPP in the study area. Also the disease was found to be very common among the cattle during the rainy season and is one of the major infectious diseases in the state, together with Anthrax, Trypanosomosis, Black Quarter and Lumby Skin Disease. Therefore based on this study the following recommendations are suggested:

1. Short term vaccination programme, with treatment of clinical cases to minimize the mortality rate to CBPP in infected areas.
2. Strengthen annual vaccination with cattle movement control through creating awareness about the disease amongst the livestock keepers.
3. Introduction of stamping out policy to minimize the infection rate of CBPP in endemic areas. (This involved test and slaughter and it can be applied, with compensation and more dialogues with livestock communities).

4. Improvement and strengthening of public and private veterinary service delivery to improve mitigation of risks imposed by CBPP.
5. The use of Community-Based Animal Health Workers that has showed a significant impact in the pastoral community should be encouraged.
6. Training of field staff in the clinical recognition of the disease and pathological differentiation from other types of bovine pneumopathies is very important.
7. The potency assessment test of CBPP vaccines that were recently used in South Sudan is highly recommended.

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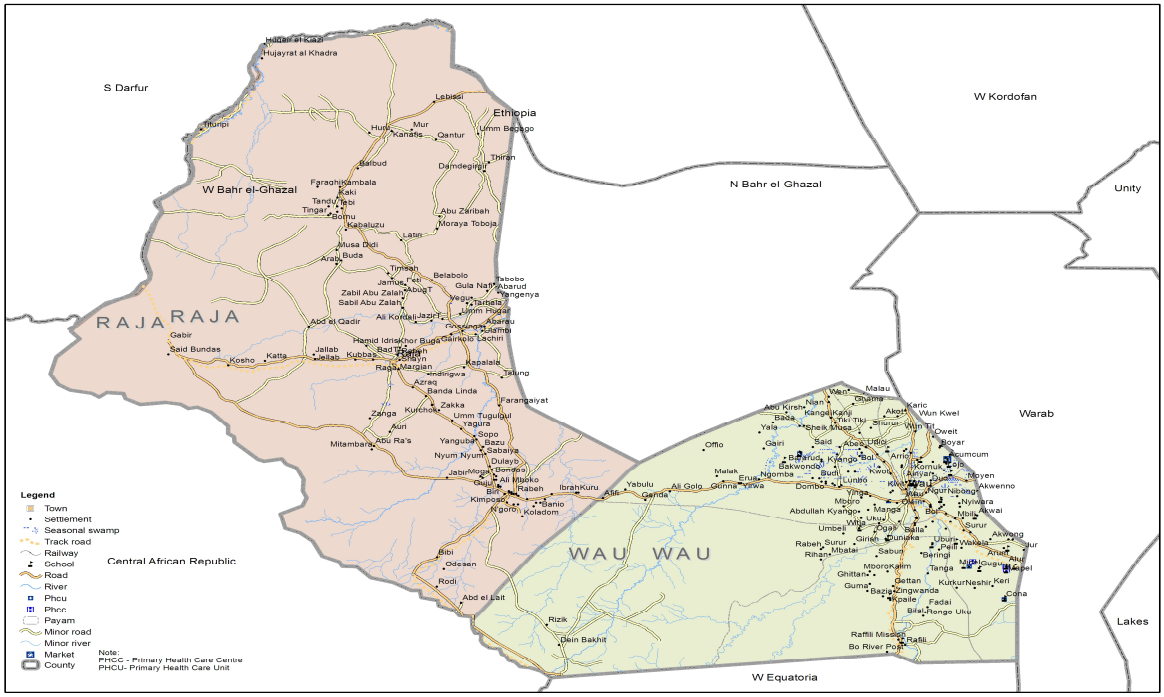
ANNEX

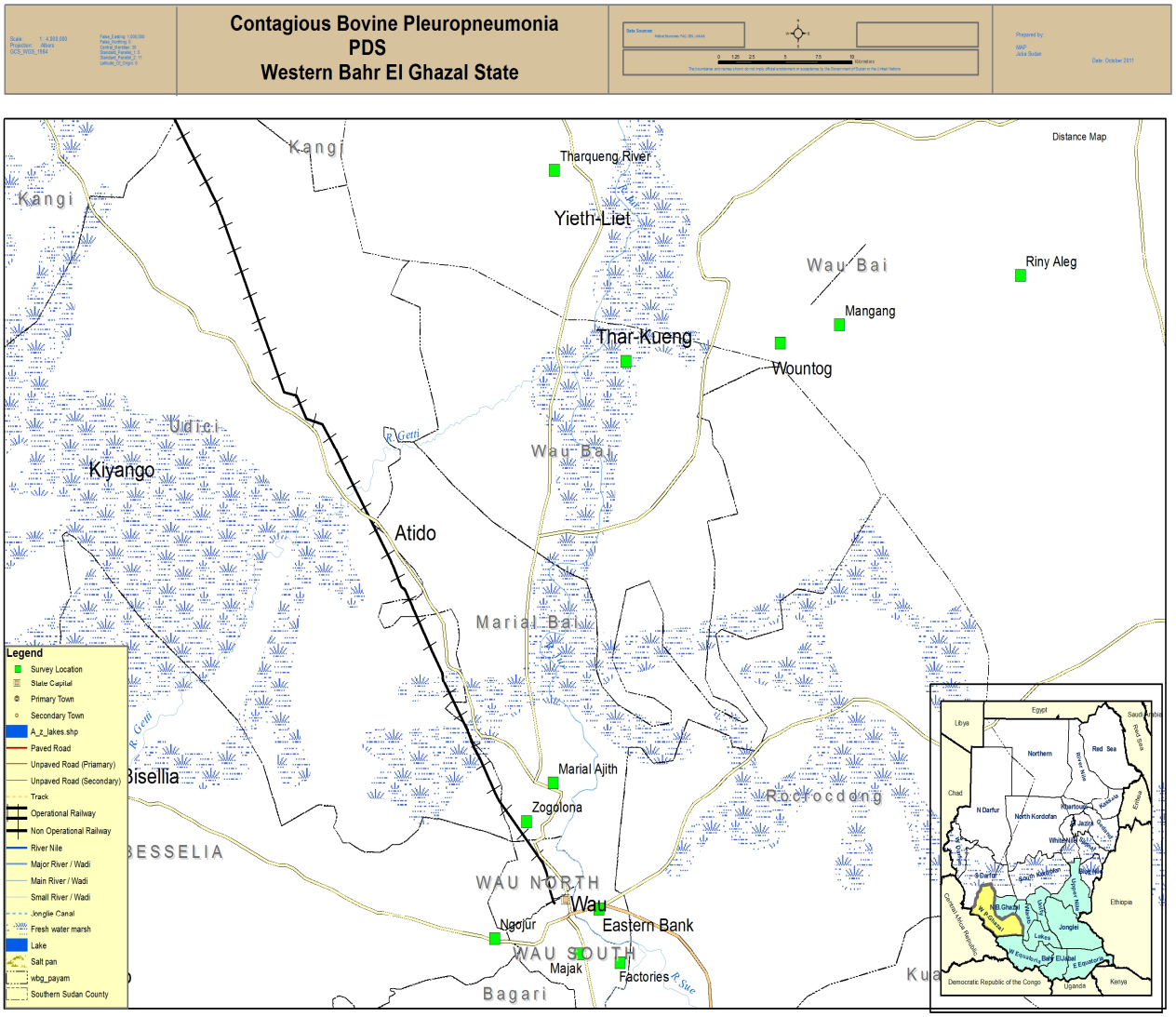


Annex1: Map of Western Bahr El Ghazal State

Annex 2: Nilotic Breed in Majak Cattle Camp- Wau

Annex 3: Proportional Piling in Eastern Bank Cattle Camp

Annex 4: Collection of blood sample from jugular vein, by vacutainer tube.

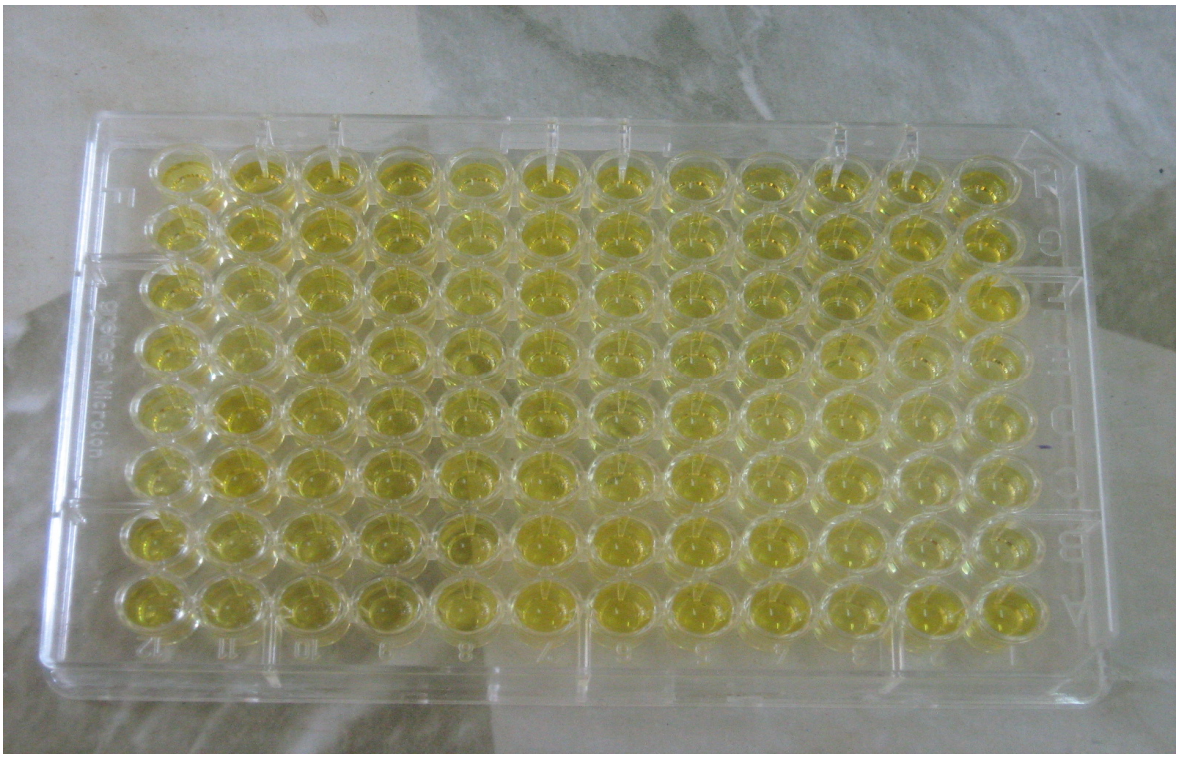


Annex 5: Map showing different locations covered during field research

The green dotes in the map represented the area covered during the field research in two counties of Western Bahr El ghazal State (Jur River and Wau County).

Annex 6: Sera, conjugate control, monoclonal control, strong positive, weak positive, negative control, pre-plate

Annex 7. Dispensing the dilution buffer in the wells of the pre-plate



Annex 8: Coated plate, final step of c-ELISA test after Revelation solution was added and ready for reading by computer

Annex 9: Contagious Bovine Pleuropneumonia Participatory Disease Search Interview Record

PDS techniques used: Semi-structures interviews, proportional piling and seasonal calendar.

1. General Information

Interview number:	State:	County:
Date:	Payam:	Location of interview:
GPS Co-ordination:	Latitude:	Longitude:
Time:	No. of informants:	Ethnic group:
Names of main informants:		

2. Types and approximate number of livestock kept:

3. Current problems in the herds

4. Most five (5) important cattle disease in the herd

Local name	Main clinical signs	English translation	Result of ranking/scoring	

Result of probing: _____

5. **Details of CBPP in the area**

- Local name of CBPP
- Year of last CBPP outbreak in the area
- Location of outbreak
- Clinical sign seen
- Cause or origin of outbreak
- Number of cattle affected
- Total population of affected herd/cattle camp
- Was outbreak seen by informant?

6. Result of observation of cattle herd/camp

Approximate number of cattle:

Disease/clinical sign	Number of cattle affected

7. Seasonal calendar

Diseases	Seasons			
	Mayi (January-March)	Yakthok (April-May)	Keer/Ruowl (June-October)	Apuok Pai (November-December)

Annex 10: Contagious Bovine Pleuropneumonia Sampling Form

State:_____ _**County**:_____**Payam**:_____ **Date**: _____

Name of cattle camp/village:_____ **Name of Investigator**:_____

Position of investigator:_____**GPS Co-ordinates**: _____

S/ No.	Sex.	Owners name	Name of animal (or description)	Age	Group
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					

Annex 11: Instructions for use of c-ELISA

1) **DEPOSIT OF THE SERA**

Prior to transfer them in the micro-plate, the samples to be analyzed are diluted and incubated with the monoclonal anti-MmmSC antibody (Mab) in a pre-dilution plate with 96 wells, hereafter called “pre-plate”. This pre-plate should be made out of normal plastic without any absorption activity.

a) **Dilution of the sera**

These dilutions must be carried out just before the test.

Dispense 100 Þl “Dilution buffer 24” in all the wells of the pre-plate

Dispense 11 Þl of the 3 control samples: CP++ in B1, B2, C1, C2 and CP+ in D1, D2, E1, E2 and CN in H1, H2

Dispense 11 Þl “Dilution buffer 24” in the wells A1, A2.

Dispense 11 Þl of the samples to test in the other wells (A3 to H12)

b). **Reconstitution and deposit of the monoclonal antibody (Mab)**

Reconstitute the Mab with 1 ml of distilled water. Dilute the necessary quantity of the Mab to 1/120 in “Dilution buffer 24” (for example 100 Þl Mab for 11.9 ml “Dilution buffer 24” for one plate).

Dispense 110 Þl “Dilution buffer 24” in the wells A1 and A2

Dispense 110 Þl diluted Mab in the other wells except A1 and A2, which are hereafter called conjugate control (Cc).

Note: If the whole amount is not immediately used, the monoclonal antibody must be stored in aliquots at a temperature < - 16 o C (before dilution in “Dilution buffer 24”).

c) **Incubation of the mix serum / monoclonal antibody**

Transfer 100 Þl of this mixture sample / Mab from the pre-plate to the control plate (see fig. 1 below) by using a multi-channel pipette. Incubate the plates 1 hour at 37o C (±3 o C) under gentle agitation.

Figure 15. Distribution of sera and Mab

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1	2	3	4	5	6	7	8	9	10
B	CP++	CP++	11									
C	CP++	CP++	21									
D	CP++	CP++	31									
E	CP++	CP++	41									
F	Cm	Cm	51									
G	Cm	Cm	61									
H	CN	CN	71									80

Cc: Conjugate control (without serum, without Mab = 100 % inhibition)

Cm: Monoclonal control (without serum = 0 % inhibition)

CP++: Strong positive serum

CP+: Weak positive serum

CN: Negative serum

1: Serum no 1

2: Serum no 2

3: ……

2) **WASHING**

a) Dilute a vial of “Wash concentrate (20x)” in 1900 ml of distilled water. This solution is hereafter called “wash solution”. The dilution can be carried out before the disappearance of the crystals which previously appeared at + 21°C (± 5°C) as long as the whole 100 ml vial is used.

b) Empty the content of the plate by returning or by another manual or automatic method.

c) Fill all the wells of the plate with the wash solution; and then empty them again.

d) Repeat step c) (a total of 2 washes).

3) DEPOSIT OF THE CONJUGATE

a) Dilute the conjugate to 1/100 in “Dilution buffer 24”

b) Dispense 100 Þl of diluted conjugate in each well.

c) Cover the plate (with a lid or aluminum foil or adhesive) and leave to incubate for 30 minutes (± 3 min) at 37 o C (± 3 o C) under gentle agitation.

4) WASHING

a) Empty the content of the plate by returning or by another manual or automatic method.

b) Fill all the wells of the plate with the wash solution; then empty them again.

c) Repeat step b) twice (a total of 3 washes)

Notes:

The care brought to the last washing is essential for a good implementation of the test. If the washing is manual, it is possible, after the last washing, to tap the plate on an absorbent towel in order to empty the wells completely.

5) **REVELATION**

a) Dispense 100 Þl of “Revelation solution.